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**14. ABSTRACT**

SteriFx has a proprietary COTS technology that has a high potential to serve as a mass decontamination chemical that is very safe and consists of all GRAS components. Previous work has demonstrated efficacy against spores, and this study was conducted to: confirm the safety of the product, better understand the interaction of the product with common military and first responder equipment/vehicles, and the capacity of the technology to inactivate viable spores of threat agents. There were no unexpected results that would make the technology unusable on equipment/vehicles, and that the product is no more corrosive than common solutions used in decontamination scenarios. The efficacy of the formulation in decontaminating anthrax spores is promising as it represents a truly safe (for human skin contact) decontaminating solution for mass casualties. Work is planned to strengthen the sporicidal activity of the formulation, test in the presence of an organic load, demonstrate activity on a human skin model, and to begin field trials.

**15. SUBJECT TERMS**
Decontamination, COTS technology; sporicidal; mass casualty; mass decontamination; civilian decon

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**INTRODUCTION:** Traditional technologies available for mass decontamination have several limitations for personnel use. Fumigants are precluded for human use due to their high toxicity. Water-based decontamination technologies (e.g. soap and water) facilitate removal of the biological agent from skin, but have little effect on the viability of organisms in the run-off resulting from the process. Bleach (hypochlorite) is an efficacious biological disinfectant, but its use on personnel is discouraged due to harsh chemical effects on the skin and respiratory tract. Modec foam, not originally designed for human use, contains strong oxidants that can harm skin and eyes. A safe, easily disseminated and effective alternative biological decontamination agent is needed to address mass personnel decontamination needs and minimize runoff waste disposal concerns. SAIC has joined with SteriFx to address the problem of mass decontamination. This partnership has the combined ability to provide the DoD with SteriFx COTS disinfectant production capacity and applications experience with SAIC's efficacy testing and user training capabilities to meet Joint Forces biological defense requirements. SteriFx has already developed a low-cost, lightweight, single-use individual product for field self-decontamination. Surface (skin) decontamination of Bacillus subtilis spores has also been documented for these products (McKillip and Dankert, 2003). Safe use of SteriFx products on military and first responder personnel in a field spray decontamination unit was previously demonstrated in 2003 at Guantanamo Bay, Cuba (Dankert, 2003), and for wound healing with repeated application over several days (Dankert, 2002). If the SteriFx decontaminant preparation is efficacious against biological agents, then it could be used directly in a shower system or water bottle application to decontaminate large groups in the event of a BWA attack. Since SteriFx is already a decontamination preparation, then runoff from such a decontamination event poses a greatly reduced chance of spreading the BWA from spilled runoff and simplifies disposal of the runoff left after a decontamination event.

The proposed study sought to address time-dependant efficacy testing of the SteriFx decontamination preparation in solution (to represent runoff disinfection) and on the surface of cultured human skin (to reflect personnel decontamination) with avirulent *Bacillus anthracis, Yersinia pestis and Francisella tularensis* as the BWA stimulants. Logistical complications due to loss/changes in personnel at SAIC, and shift of units within SAIC to perform the studies, led to a no-cost extension request for the actual study. Permission for the extension was granted, and re-negotiating subcontract with the new SAIC business unit was required.

Laboratory testing by SAIC will evaluate the bactericidal/sporicidal efficacy of SteriFx preparations (DeconFx) in solution with Bacillus anthracis delta Sterne

This study sought to determine how long SteriFx Solutions must be in contact with the simulants in solution for disinfection, an indicator of how long the SteriFx runoff from personnel decontamination is still biologically hazardous. Spores and bacterial cells will be prepared for the decontamination experiments. In the case of spores, the %spores in the preparation will be determined to assure that

the % spores in the solutions used in these studies are high (e.g. $>10^7$/ml). The solution decontamination efficacy of SteriFx products will be evaluated first to determine the log kill versus contact time with SteriFx products in aqueous solutions at room temperature incrementally from 0 to 60 minutes to simulate runoff from human decontamination activities. After the specified SteriFx contact time, SteriFx will be neutralized with buffer to stop the decontamination process and the bacteria/spores recovered by centrifugation. The bacteria/spores will be washed, then dilution plated on agar medium to enumerate them. Untreated controls and samples of the culture/spore preparation will also be enumerated concurrently to allow comparisons of the bacteria/spores applied and recovered. The log kill associated with incremental contact times with SteriFx will establish the minimum contact time required in solution. This information will be used to design the surface contact decontamination experiments for the next study task, cultured human skin decontamination of BWA stimulants found susceptible to SteriFx disinfection.

Cultured human skin decontamination was not addressed due to lack of time and change of SAIC labs (no working skin decon model available).

Log kill data from both solution and skin surface studies of the stimulants will be compared to evaluate the disinfection efficacy of the technology on bacterial cells and spores. The minimum time to reach the maximum log kill of the bacteria/spores in solution and on skin surface will be determined from the assembled data. The ability of SteriFx to achieve a complete log kill of the stimulants will also be determined from the study results. This information will be incorporated into the final report conclusions and recommendations made regarding applicability to mass decontamination scenarios and future disinfection studies (pending further funding) with virulent pathogens in a BL3 facility and studies with interferents likely encountered in a mass decon scenario (e.g. sunscreen, lotion, dirt, etc.).

**BODY:**

The project has suffered a delay in execution, as the principal investigator/project director for SAIC, Ilona Fry, passed away. This set-back has caused a delay in all aspects of the project including scheduled laboratory time designated for the project. All principals with SAIC have been re-assigned during this delay, and SteriFx has been working with SAIC to establish a new team on the part of SAIC. In building the initial coalition with SAIC, SteriFx worked for over two years to garner the attention of SAIC for the project, and to bring all participants up to date on the SteriFx technology. This re-contact and training has been slower than expected, and is the reason SteriFx requested the 1 year extension on the project. Juanita Livingston, Technical Editor Information Management at Fort Detrick, MD (301-619-7325) has been assigned to oversee the extension.

Targeted Tasks:

Establish feasibility of CleanseFx/DeconFx to reduce bacterial loads in contaminated wounds.

Determine effect of CleanseFx/DeconFx on animal model tissues.

Measure the effect of Fx Technology on common metals and other substances. Measure weight loss of sample metals incubated in SteriFx Technology.

Determine any detrimental effects of Fx Technology on plastics, rubber and plastic tubing, and clothing.

Demonstrate ability of CleanseFx to function as skin cleanser in military setting. Provide Military Field Users with solutions for evaluation.

Demonstrate ability of CleanseFx to function as skin cleanser in military setting. Obtain guidance from users on benefits of CleanseFx in practice.

Much of the work outlined is dependent upon the use of high-level targets (bacterial spores) to be neutralized by SteriFx technology. The proposed study addressed the time-dependant efficacy of the SteriFx decontamination preparation in solution (and to represent runoff disinfection questions as well). These targets and techniques are dependent upon facilities not available at SteriFx, but can be addressed in the SAIC facility. The work is dependent upon the cooperation between SteriFx and SAIC, and we have been working to accomplish this after the first SAIC team had been re-assigned.

SteriFx has utilized corrosion testing and has some preliminary findings on compatibilities. These results, submitted previously, are added as Appendix 1 to this document.

**Studies of SteriFx DeconFx Sporicidal Efficacy against *Bacillus anthracis* Spores**

Experimental Plan

Laboratory analysis was initiated by SAIC/CHORI to test the sporicidal efficacy of SteriFx decontamination preparations (DeconFx) in solution with avirulent *Bacillus anthracis* Sterne 34F2 spores – a representative test case for the efficacy of DeconFx against the causative agent of Anthrax. This study was designed to determine the required DeconFx contact time and concentration to achieve a six log reduction in spore viability (sterilizing decontamination) in solution, an indicator of how long the SteriFx runoff from personnel decontamination would be biologically hazardous. Spores were prepared by methods established at CHORI for previous decontamination studies including >95 % spore viability and $>10^7$ colony forming units/ml. DeconFx, and DeconFx concentrate, was prepared at SteriFx and shipped to the SAIC Laboratories. Validation of efficacy against low-level vegetative pathogens was performed at SteriFx laboratories. The efficacy and physical properties of the solutions were validated (results presented in Appendix 2).

The decontamination efficacy of SteriFx products is being evaluated to determine the log kill versus contact time with DeconFx products in aqueous solutions at room temperature (rt) and 37°C to simulate runoff from human decontamination activities. At each DeconFx contact time series sample point, DeconFx is neutralized with NaOH to stop the decontamination process. The spores are then plated onto culture media to determine the number of remaining viable spores. Untreated controls and samples of the spore preparation are also be enumerated concurrently to allow comparisons of the spores applied and recovered. The log kill associated with incremental contact times for DeconFx products establishes the minimum contact time required in solution for sterilizing decontamination. This information will be used to design surface contact regimes that can be utilized in future simulated and real-world decontamination tests.

Following initial studies to assess if DeconFx had bioactivity against *B. anthracis* spores (see previous report), an extended time course study was designed to determine if DeconFx had sterilizing *B. anthracis* sporicidal activity (six logs spore kill) under well controlled and established experimental conditions. The study was conducted using very high levels of inoculum ($>10^7$) of bacterial spores.

Experimental Methods for Normal Concentration (RTU) DeconFx Study

Spore titer was determined by serial dilutions (sterile deionized water) that were plated onto Tryptose Blood Agar Base (TBAB) agar plates at 37°C. Initial spore stock titers were in the range of $10^8$ heat resistant (60°C for thirty minutes) spores/ml.

The following test protocol was used.

1. 1 to 2 x $10^7$ spores were added in a 295 µl aliquot to 5 ml falcon tubes.
2. 1.0 ml of DeconFx (RTU) was added to each tube and incubated for the times indicated (this reduces concentration of the sporicide to 77% of starting strength). Experiments were conducted at room temperature and at 37°C.
3. At the end of incubation, 0.380 mls of 10 N NaOH was added to each tube on ice to neutralize DeconFx (resulting pH was ~6.0).
4. Each tube was serially diluted, plated onto TBAB agar, incubated overnight at 37°C, and viable counts determined to quantify the number of spores remaining after treatment.
5. For each experiment, a positive control received the same spore input, was re-suspended in neutralized DeconFx, and plated to determine the actual number of spores aliquoted in each experiment.

Results

The results from the extended time course sporicidal efficacy study of normal strength DeconFx conducted at 20° (rt) and 37°C are shown in Figure 1.

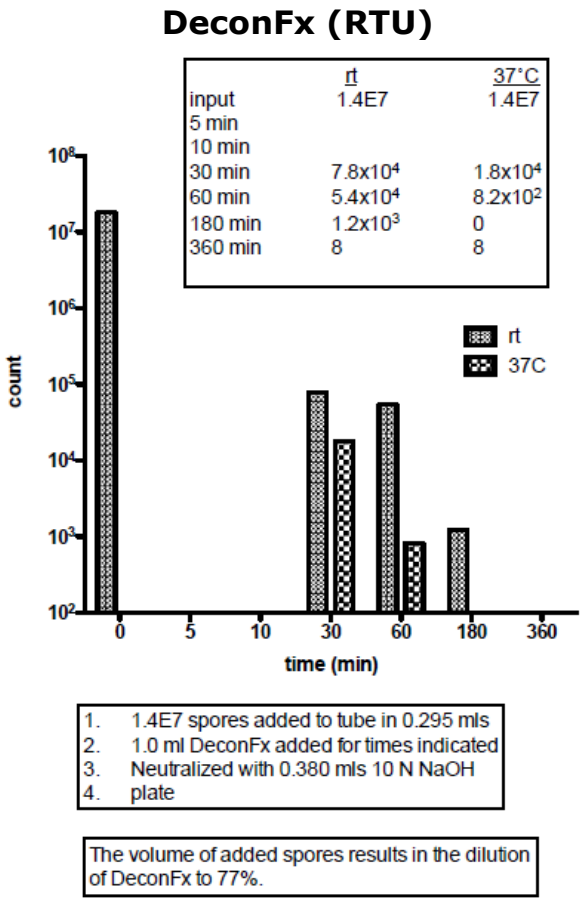



**Figure 1**

These data demonstrate that normal strength DeconFx does have the ability to kill highly concentrated populations of *B. anthracis* spores with sufficient contact time. The spore inactivation rate is accelerated at 37°C and appears to be a first order (exponential) process with no observable lag time. These results have been obtained in two independent experiments. With three hours of contact time, DeconFx is able to achieve six logs of spore kill (sterilizing decontamination). These experiments also demonstrate that normal strength DeconFx is stable and active over three hours of contact time with spores.

Positive controls which received the same spore input as the test samples, were re-suspended in neutralized DeconFx, and plated to determine the actual number of spores aliquoted in each experiment. There was no difference between the spore titers of the positive controls and the expected titer calculated for experimental dilution of the original spore stock.

Experimental Methods for the Concentrated DeconFx Study

A concentrated DeconFx preparation (approaching the solubility limit for the active components) was evaluated to determine the maximal sporicidal effects that could be achieved with this product. Spore titer was determined by serial dilutions (sterile deionized water) that were plated onto Tryptose Blood Agar Base (TBAB) agar plates at 37°C. Initial spore stock titers were in the range of $10^8$ heat resistant (60°C for thirty minutes) spores/ml.

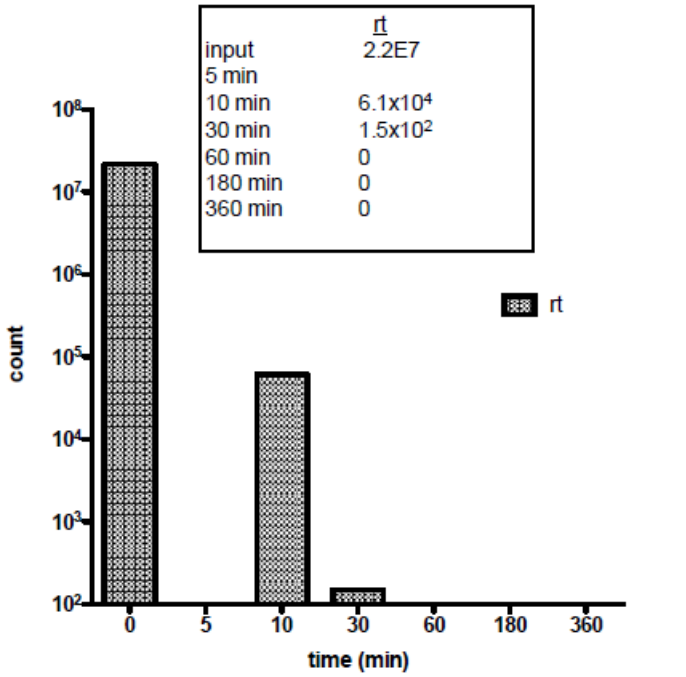
The following test protocol was used.

1. 1 to 2 x $10^7$ spores were added in 295 µl to 5 ml falcon tubes.
2. 1.0 ml of concentrated DeconFx was added to each tube and incubated for the times indicated (this reduces concentration of the sporicide to 77% of starting strength). Experiments were conducted at room temperature and at 37°C.
3. At the end of incubation, 0.880 mls of 10 N NaOH was added to each tube on ice to neutralize DeconFx (resulting pH was ~6.0).
4. Each tube was serially diluted, plated onto TBAB agar, incubated overnight at 37°C, and viable counts determined to quantify the number of spores remaining after treatment.
5. For each experiment, a positive control received the same spore input, was re-suspended in neutralized DeconFx, and plated to determine the actual number of spores aliquoted in each experiment.

Results

The results of the extended time course concentrated DeconFx sporicidal efficacy study conducted at 20° (rt) are shown in Figure 2 below.

## Concentrated DeconFx




1. 2.2E7 spores added to tube in 0.295 mls
2. 1.0 ml Concentrated DeconFx added for times indicated
3. Neutralized with 0.885 mls 10 N NaOH on ice
4. plate

The volume of added spores results in the dilution of concentrated DeconFx to 77%.

**Figure 2**

These data demonstrate that concentrated strength DeconFx has enhanced ability to kill high density populations of *B. anthracis* spores even at room temperature. Previous studies with normal strength Decon Fx demonstrated that the DeconFx inactivation rate was diminished at 20°C when compared to 37°C kill rates. Concentrated DeconFx spore inactivation at 20°C appears to be a first order (exponential) process with no observable lag time. These results have been obtained in two independent experiments.

Positive controls which received the same spore input as the test samples, were re-suspended in neutralized DeconFx, and plated to determine the actual number of spores aliquoted in each experiment. There was no difference between the spore titers of the positive controls and the expected titer calculated for experimental dilution of the original spore stock.

With one hour of contact time, DeconFx was able to achieve six logs of spore kill (sterilizing decontamination). These experiments also demonstrate that concentrated DeconFx was able to completely sterilize high density *B. anthracis* spore populations with contact times similar to strong

oxidizing agents such as concentrated bleach (sodium hypochlorite) or hydrogen peroxide which are substantially more hazardous, toxic and corrosive than concentrated DeconFx.
In addition, bleach exhibits a lag time in initiating exponential spore killing which is not observed with DeconFx. These studies also demonstrate that DeconFx spore killing rates are concentration and contact time dependent which allows modeling of DeconFx sporicidal bioeffects.

The data obtained from these and previous studies suggest that concentrated DeconFx is a very promising sporicide that merits further investigation as a next-generation sporicidal decontaminant. The safety of this concentrate needs to be firmly documented.

**RESEARCH ACCOMPLISHMENTS:**

1) Re-establish teaming with SAIC in order to complete tasks against high-level bacterial targets.

2) Demonstrate the efficacy of DeconFx formulations to kill bacterial spores:

    a. DeconFx safety in terms of exposure to human skin can be coupled with this spore-killing information as the basis to produce a high-level sporicidal decontaminating system for personnel and mass casualties.

    b. DeconFx can be tested further to establish the technology as a cold sterilant.

**REPORTABLE OUTCOMES:**

The inability to begin the project on time has resulted in a number of set-backs including scheduling of laboratory time, re-assignment of personnel for the project, and training of new personnel. Tasks accomplished include those dealing with non-bacterial targets, such as the necessary identification of certain substances, common in military or first-responder equipment and vehicles. The reports to date indicate that the technology should be treated as any mildly acidic compound, and that degradation of concrete, discoloration of soft metals, dissolution of electrical contacts are a possibility. However, there are no unexpected results that would make the technology any more of a concern than this. It should be noted that even bleach solutions, common in decontamination scenarios, can have a similar corrosive potential.

In terms of the spore killing results here, the goal of producing a safe and effective decontaminating solution that is currently available (COTS) is now more evident. The spore-killing demonstrated previously by SteriFx on an animal skin model (McKillip and Dankert, 2003), and in other studies have been confirmed here using anthrax spores. The ability of low levels of DeconFx and short

exposure times argues that even if not used as a cold sterilant for personnel decontamination, that short exposure times could result in a 99% decrease in infective dose of a person exposed to spores. This would be a very useful tool for medics and first responders handling casualties or exposed wounded in any emergency event. The chances for spreading the agent further are drastically reduced. The technology offers this potential, and future studies will be directed to:

1. Augmenting the efficacy of DeconFx formulations by adding additional components and by formulating higher concentration stocks of existing formulation.
2. Define the effects of organic load on efficacy.
3. Characterize the ability of DeconFx to kill spores on various surfaces (glass, fabric, etc).
4. Provide a skin model system to perform spore killing tests.
5. Test formulations for field use.

**CONCLUSION:** SteriFx technology continues to be in use to control pathogens in large industrial settings for the food industry, even to being approved as a food ingredient. All components of DeconFx are GRAS (generally recognized as safe) by the FDA, and this has been confirmed by the FDA. The DeconFx concentrate, used in these studies, is the same formulation and concentration that has been provided to the food industry, and used by them, since 2004. The concentrate is diluted over 100 times for the food safety applications (less than 1% strength) to kill Salmonella, E. coli, etc. The concentrate is handled/manipulated by plant personnel without requiring special ventilation or storage conditions. The systems in use in these industrial applications can be easily transferred to mass casualty applications. This potential, as outlined in our preliminary work, shows that the technology has a very low risk of doing harm to personnel in decontamination scenarios, or that misuse of the technology would pose harm. The potential to provide an anti-sporicidal agent that can be used easily by the military and first responders, to include the civilian population in mass terror or casualty events, is reason to pursue and complete this project.

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**APPENDICES:**

Appendix 1: Corrosion Report.

Appendix 2: DeconFx Validation.

Appendix 3: Initial Studies of Sporicidal Efficacy against *B. anthracis* Spores.

Appendix 4: SteriFx Concentrate MSDS

## Appendix 1:

Compatibility of DeconFx Decontamination Solution at Various pH Levels to Materials (W81XWH-BAA08-1)

John R. Dankert, Ph.D.
SteriFx Inc.

DeconFx is a tri-acid mixture that has shown, in spite of its low pH, strong compatibility with animal tissue. In each area for consideration, skin, inhalation, ingestion, and eyes, the product test results fall in the lowest risk group, making it consistent with the GRAS (Generally Regarded As Safe) status of the product components.

DeconFx and other products in the same technology family are acids and will function as such on materials corroded or attacked by acids. Metals that are susceptible to corrosion, polymers attacked by acids, or basic materials could be affected by DeconFx. The susceptibility of various materials to DeconFx was measured under accelerated conditions following the ASTM G-31 protocol. Sample results are shown below and are expressed as a corrosion rate of "mils per year", one mil = 0.001 inches. The ability for DeconFx to attack metals depends on the pH of the solution and the metal in question.

| | pH | | | | | |
|---|---|---|---|---|---|---|
| | 0.4 | 1 | 2 | 3 | 4 | 5 |
| Carbon Steel P-3 | 260 | 140 | | | | |
| Stainless Steel 304L | | <1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Stainless Steel 316L | | <1 | | | | |
| Copper 110 | 390 | | | | | |
| Brass 443 | 290 | | | | | |
| Brass 510 P | 320 | | | | | |
| Aluminum 1100 | | 290 | 27 | 3 | 4 | 5 |
| Aluminum 7075 T-6 (aircraft) | rapid | | | | | |

DeconFx is fully compatible with several common polymers. These polymers were tested under accelerated conditions following the ASTM G-31 protocol. Accelerated conditions involve elevated temperatures that can thermally produce degradation of the polymer irrespective of the DeconFx test solution. The potential for thermal degradation was separated from any effect the DeconFx solution had on the polymer in these tests by exposing blank samples to the same heat history profile as the DeconFx exposed samples.

In the table below are found sample results expressed in percent change in hardness, a measure of degradation. Nylon is exceptionally unsuitable in contact with DeconFx. It is a polyamide resin based product and should not be exposed to DeconFx. Polyamide resins react with the acids present in DeconFx and dissolve under ambient conditions. Likewise, polymers filled with acid reactive materials, like calcium carbonate, should be avoided for contact with DeconFx. These polymers may be structurally degraded as the acid potentially leaches calcium carbonate from the polymer, leaving voids behind.

| | pH | | | | | |
|---|---|---|---|---|---|---|
| | 0.4 | 1 | 2 | 3 | 4 | 5 |
| Butyl Rubber | -0.4 | | | | | |
| Viton | 1.3 | | | | | |
| PVC | -0.9 | | | | | |
| HDPE | 1.8* | | | | | |
| PP | 0.0* | | | | | |
| Teflon | -3.7 | | | | | |

* Test done on concentrate as pH = -0.4

The results presented above correspond to continuous exposure of DeconFx solution to the material at the indicated pH. DeconFx contact during actual use with metals that otherwise are susceptible to corrosion require a water rinse following exposure.

Storage Tanks

Storage tanks used with DeconFx concentrate must meet the chemical resistance requirements for dilute hydrochloric and dilute phosphoric acids. A typical storage tank material approved for FreshFx and suitable for storing materials intended for use in food contact is linear polyethylene. For food contact application, the tank manufacturer must certify that the tank meets the specifications contained in FDA regulation 21CFR177.1520 (c) 3.1 and 3.2.

Filling a storage tank is done through a 2 inch diameter hose supplied with the tank truck. The loading pipe to the storage tank needs to be equipped with a male 2 inch PVC CAM lock fitting to facilitate connection with the truck hose. The truck off-loads the product using air at 10 psig. The pipe leading to the storage tank must be securely attached to supporting members or the tank to handle the vibration encountered when the air pressure from the truck "blows off" at the end of the transfer. During the transfer, the storage tank must be vented to accommodate the sudden increase in air volume from the filling pipe at the end of the transfer.

## APPENDIX 2:

### DeconFx Validation

SteriFx C-12 concentrate was used to make DeconFx for testing at SAIC Labs. Concentrate was manufactured by Brainerd Chemicals, Tulsa, OK. The density of the concentrate was 1.23 gm/mL and conforms to previous density records. Dilution vs pH testing was done as to verify the acidic strength of the concentrate. DeconFx was prepared by diluting the concentrate with deionized water (1 vol conc: 2 vol water). Figure 1 below represents Dilution vs pH Value of various dilutions of the prepared DeconFx into deionized water (DiW).

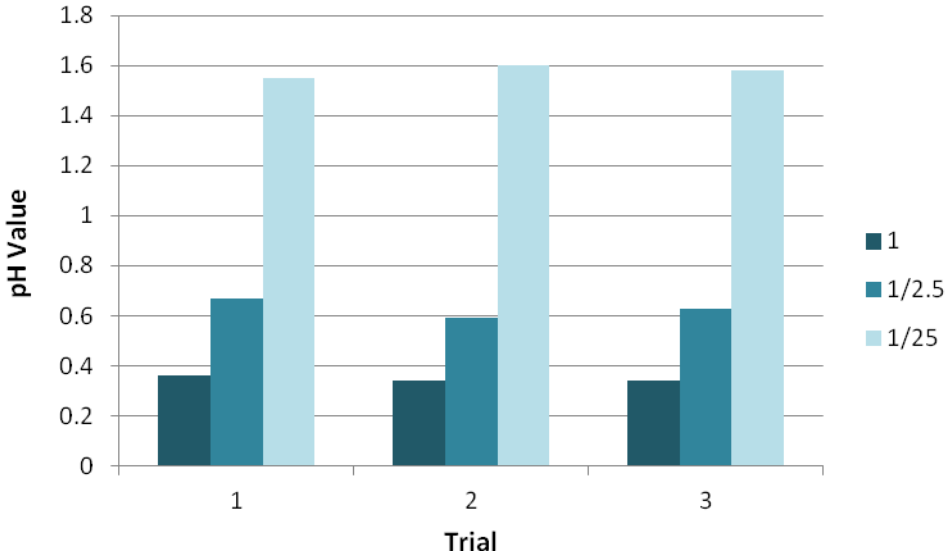


*Figure 1. The measured pH value of various concentrations of DeconFx diluted into DiW. Legend represents the undiluted DeconFx (1), and a 2.5x and 25x dilution of the DeconFx. All dilutions and recordings were taken at RT.*

The prepared DeconFx was used to inhibit the viability of vegetative cells to establish efficacy. Salmonella typhimurium and Escherichia coli (Gram positive and Gram negative, respectively) were used as target organisms.

Materials and Methods. Cultures of bacteria were inoculated into Peptone broth and incubated overnight at 37° C the day before experiments were performed. Cultures were used without washing prior to assays. Agar plates were used for enumeration.

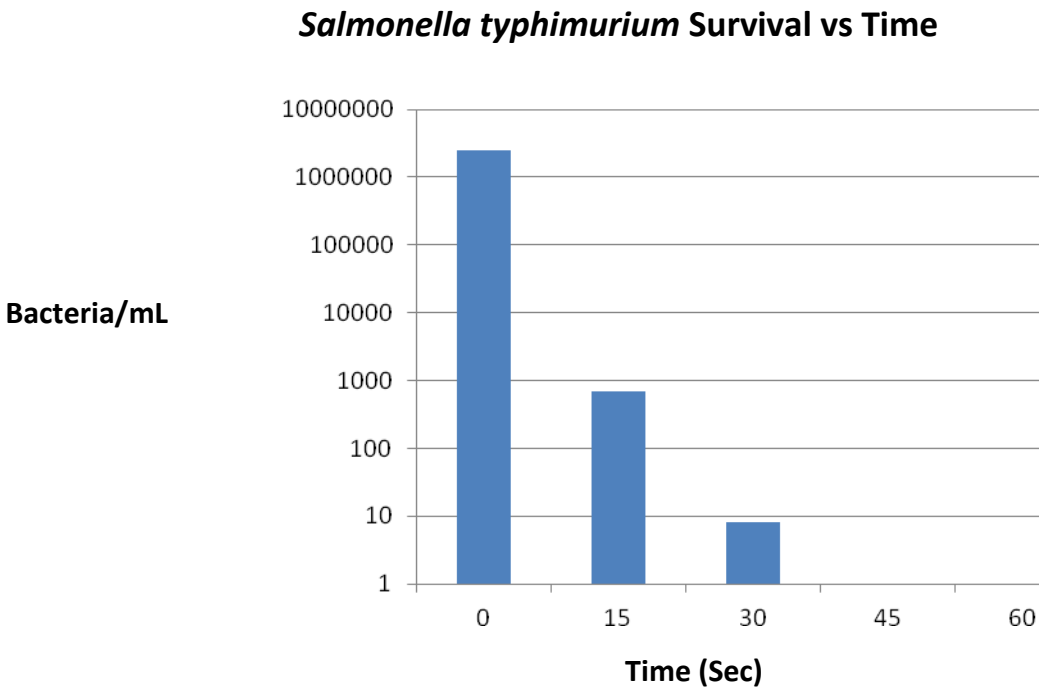


*Figure 2. S. typhimurium cells grown as described in Materials and Methods were placed into an equal volume of DeconFx that had been previously diluted 1 to 1 in deionized water (DiW), making an effective final concentration of DeconFx ¼ strength of the RTU solution (or, 0.25x). All solutions were allowed to equilibrate to room temperature (RT) prior to mixing. For the t=0 time point, a separate aliquot of cells were placed into an equal volume of DiW. The mixture was incubated at room temperature with occasional shaking, and aliquots were removed at the indicated times and placed into 9 volumes of Peptone Broth. These solutions were then serially diluted in Peptone Broth for plating. 100 µL aliquots of the serially diluted plates were spread on TSA plates, and incubated for 16-24 hrs for enumeration.*

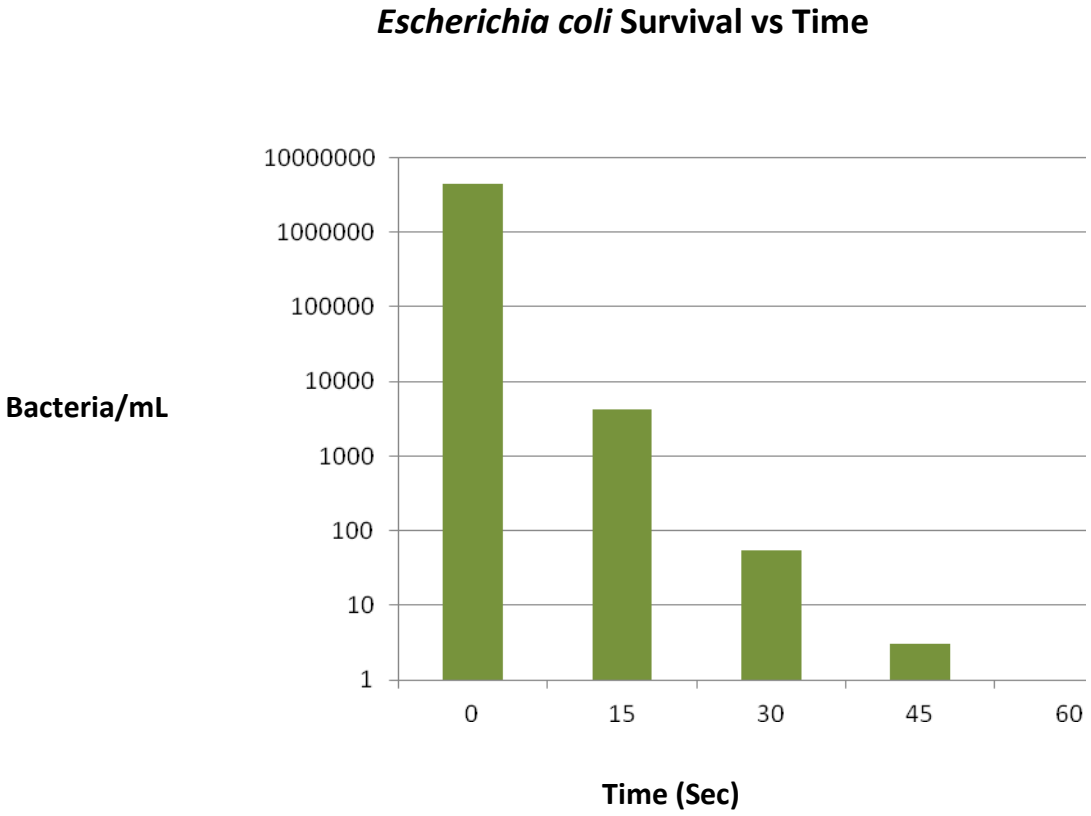


*Figure 3. E. coli cells grown as described in Materials and Methods were placed into an equal volume of DeconFx that had been previously diluted 1 to 1 in deionized water (DiW), making an effective final concentration of DeconFx ¼ strength of the RTU solution. All solutions were allowed to equilibrate to room temperature (RT) prior to mixing. For the t=0 time point, a separate aliquot of cells were placed into an equal volume of DiW. The mixture was incubated at room temperature with occasional shaking, and aliquots were removed at the indicated times and placed into 9 volumes of Peptone Broth. These solutions were then serially diluted in Peptone Broth for plating. 100 µL aliquots of the serially diluted plates were spread on TSA plates, and incubated for 16-24 hrs for enumeration.*

The above results indicate that DeconFx prepared for use by SAIC Labs was consistent with previous formulations in terms of efficacy against vegetative bacterial cells (Gram pos and Gram neg). For the project, it will be necessary to establish sporicidal activity, and activity against bacterial strains not available to SteriFx.

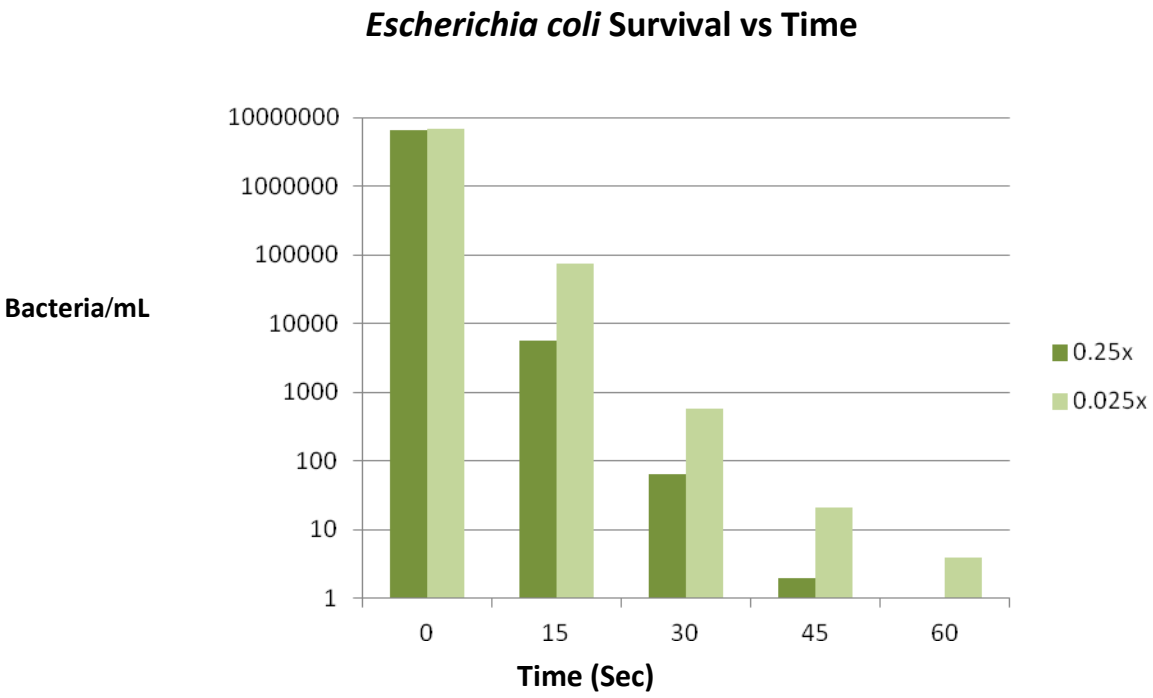


*Figure 4. E. coli cells grown as described in Materials and Methods were placed into an equal volume of DeconFx that had been previously diluted 1 to 1 (0.25x) or 1 to 10 (0.025x) in deionized water (DiW), making an effective final concentration of DeconFx ¼ strength of the RTU solution or a 1/40 strength of the RTU solution. All mixtures and solutions were allowed to equilibrate to room temperature (RT) prior to mixing. For the t=0 time point, a separate aliquot of cells were placed into an equal volume of DiW. The mixture was incubated at room temperature with occasional shaking, and aliquots were removed at the indicated times and placed into 9 volumes of Peptone Broth. These solutions were then serially diluted in Peptone Broth for plating. 100 µL aliquots of the serially diluted plates were spread on TSA plates, and incubated for 16-24 hrs for enumeration.*

This experiment demonstrates the extent of "dilution" that the formulation can endure whilst maintaining antimicrobial activity. As shown in Fig. 4 above, the formulation can be diluted over 10-fold and maintain an antimicrobial effect. For Mass Decontamination, this would be important if exposed casualty numbers exceeded capacity, where treatment regimes could be modified on a case by case basis.

## Appendix 3:

### Initial Studies of SteriFx DeconFx Sporicidal Efficacy against *Bacillus anthracis* Spores

Experimental Plan

Laboratory analysis was initiated by SAIC/CHORI to test the sporicidal efficacy of SteriFx decontamination preparations (DeconFx) in solution with avirulent *Bacillus anthracis* Sterne 34F2 spores – a representative test case for the efficacy of DeconFx against the causative agent of Anthrax. This study was designed to determine the required DeconFx contact time and concentration to achieve a six log reduction in spore viability (sterilizing decontamination) in solution, an indicator of how long the SteriFx runoff from personnel decontamination would be biologically hazardous. Spores were prepared by methods established at CHORI for previous decontamination studies including >95 % spore viability and $>10^7$ colony forming units/ml.

The solution decontamination efficacy of SteriFx products is being evaluated to determine the log kill versus contact time with DeconFx products in aqueous solutions at room temperature and 37°C to simulate runoff from human decontamination activities. At each DeconFx contact time series sample point, DeconFx is neutralized with NaOH to stop the decontamination process. The spores are then plated onto culture media to determine the number of remaining viable spores. Untreated controls and samples of the spore preparation will also be enumerated concurrently to allow comparisons of the spores applied and recovered. The log kill associated with incremental contact times with DeconFx products will establish the minimum contact time required in solution for sterilizing decontamination. This information will be used to design surface contact regimes that can be utilized in simulated and real-world decontamination experiments.

An initial scoping study was designed to determine if DeconFx had *B. anthracis* sporicidal activity under well controlled and established experimental conditions.

Experimental Methods for Preliminary Scoping Study

Spore titer was determined by serial dilutions (sterile deionized water) that were plated onto Tryptose Blood Agar Base (TBAB) agar plates at 37°C. Initial spore stock titers were in the range of $10^8$ heat resistant (60°C for thirty minutes) spores/ml.

The following test protocol was used.

1. 1 - 2 x $10^7$ spores were added in 295 µl to 5 ml falcon tubes.
2. 1.0 ml of DeconFx was added to each tube and incubated for the times indicated (this reduces concentration of the sporicide to 77% of starting strength). Experiments were conducted at room temperature and at 37°C.

3. At the end of incubation, 0.380 mls of 10 N NaOH was added to each tube on ice to neutralize DeconFx (resulting pH was ~6.0).
4. Each tube was serially diluted, plated onto TBAB agar, incubated overnight at 37°C, and viable counts determined to quantify the number of spores remaining after treatment.
5. For each experiment, a positive control received the same spore input, was re-suspended in neutralized DeconFx, and plated to determine the actual number of spores aliquoted in each experiment.

Results

The results of the initial DeconFx sporicidal efficacy study conducted at 20° (rt) and 37°C are shown in Figure 1.

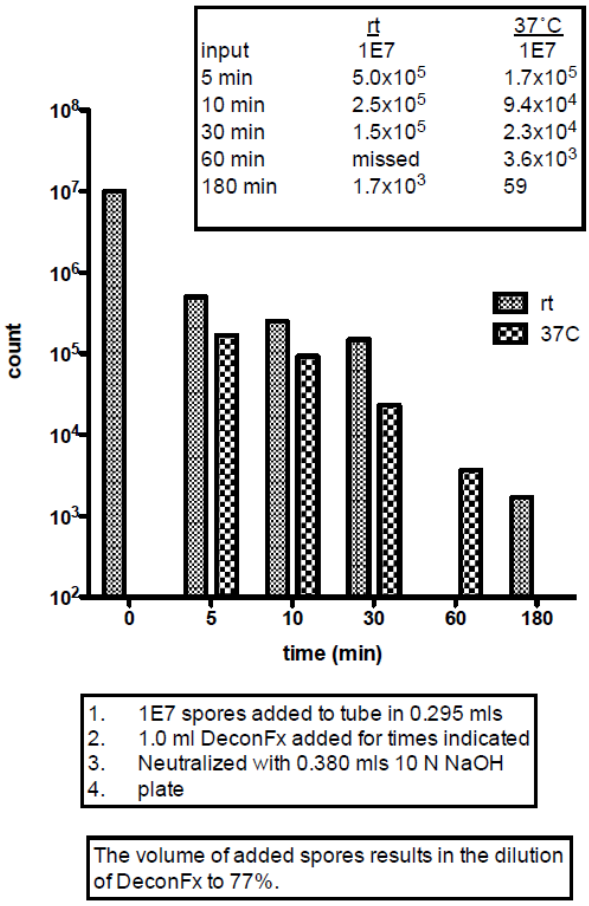


**Figure 1**

These data demonstrate that normal strength DeconFx does have the ability to kill *B. anthracis* spores. Killing is accelerated at 37°C and appears to be a first order (exponential) process with no observable lag time. These results have been obtained in two independent experiments. With three

hours of contact time, DeconFx sporicidal bioeffects approach six logs of kill (sterilizing decontamination).

Positive controls which received the same spore input as the test samples, were re-suspended in neutralized DeconFx, and plated to determine the actual number of spores aliquoted in each experiment. There was no difference between the spore titers of the positive controls and the expected titer calculated for experimental dilution of the original spore stock.

Further experiments were directed toward decreasing the DeconFx contact times required for six logs of spore killing.

## Section 1 - Chemical Product and Company Identification

**Product/Chemical Name:** DeconFx Concentrate (C-12)
**Chemical Formula:** N/A
**Other Designations:** None
**General Use:** Decontaminating Agent
**Manufacturer:** Brainerd Chemical, Tulsa, OK
**Telephone Number:** 318-425-2515
**Distributor:** SteriFx ™, Inc., 2031 Kings Hwy, Shreveport, LA 71103
**MSDS Preparer:** John R. Dankert, Ph.D.
**EPA Registration:** EPA EST No. 008743-OK-001
**Emergency Phone:** CHEMTREC 1-800-424-9300

**HMIS**
**H** 1
**F** 0
**R** 0
**PPE**†
†Sec. 8

## Section 2 - Composition / Information on Ingredients

| Ingredient Name | CAS Number | % wt |
|---|---|---|
| Phosphoric Acid | 7664-38-2 | < 18 |
| Hydrochloric Acid | 7647-01-0 | < 7 |
| Citric Acid | 77-92-9 | < 22 |

| Ingredient | OSHA PEL | | ACGIH TLV | | NIOSH REL | | NIOSH IDLH |
|---|---|---|---|---|---|---|---|
| | TWA (8-hr) | STEL (15-min) | TWA (8-hr) | STEL (15-min) | TWA (10-hr) | STEL (15-min) | |
| Phosphoric Acid | 1 mg/m$^3$ | 3 mg/m$^3$ | 1 mg/m$^3$ | 3 mg/m$^3$ | 1 mg/m$^3$ | N/A | N/A |
| Hydrochloric Acid | 7 mg/m$^3$ | N/A | 7.5 mg/m$^3$ | N/A | 7 mg/m$^3$ | N/A | 100 ppm |
| Citric Acid | 15 mg/m$^3$ *(particulates)* | N/A | 10 mg/m$^3$ *(particulates)* | N/A | N/A | N/A | N/A |

## Section 3 - Physical and Chemical Properties

**Physical State:** Liquid
**Appearance and Odor:** Clear and odorless.
**Vapor Pressure:** 17.1 mm Hg at 20 °C
**Vapor Density (Air=1):** 1.2
**Specific Gravity (H2O=1):** 1.22
**Volatile Organic Compounds:**
**Lbs/Gal:** < 0.1
**Grams/Liter:** < 10

**Water Solubility:** Completely at 25 °C
**pH @ 20°C:** -0.3
**Boiling Point:** >240 °F or >116 °C
**Freezing Point:** -20 °C
**Percent Volatile:** 96.8
**Evaporation Rate (nBuAc=1):** < 1
**Autoignition Temperature:** N/A
**Magnetism (milligauss):** N/A

## Section 4 - Fire-Fighting Measures

**Flash Point:** > 200 °F
**Flash Point Method:** Tag Closed Cup
**Burning Rate:** N/A
**Autoignition Temperature:** N/A
**Lower Explosion Limit (v/v):** N/A
**Upper Explosion Limit (v/v):** N/A
**Flammability Classification:** Non-Flammable

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**Extinguishing Media:** Use water spray, fog, foam, dry chemical, or carbon dioxide for surrounding fire.
**Unusual Fire or Explosion Hazards:** Extreme heat or contact with common metals may liberate hydrogen gas, a flammable gas that readily forms explosive mixtures with air.
**Hazardous Combustion Products:** In extremely rare cases, thermal oxidative decomposition can produce toxic chloride and phosphorous oxide ($PO_x$) fumes.
**Fire-Fighting Instructions:** None
**Fire-Fighting Equipment:** Because fire may produce toxic thermal decomposition products, wear a self-contained breathing apparatus (SCBA) with a full facepiece operated in pressure-demand or positive-pressure mode.

## Section 5 - Stability and Reactivity

**Stability:** Stable at room temperature in closed containers under normal storage conditions.
**Polymerization:** Hazardous polymerization may occur if exposed to aldehydes, epoxides, and azo compounds.
**Chemical Incompatibilities:** Avoid contact with aldehydes, amines, amides, alcohols, azo-compounds, carbamates, esters, caustics, phenolics, ketones, epoxides, organic peroxides, sulfides, potassium permanganate, fluorine, carbides, acetate, nickel carbonate
**Conditions to Avoid:** Avoid contact with incompatibles..
**Hazardous Decomposition Products:** Toxic chloride fumes and phosphorous oxide ($PO_x$) fumes.

## Section 6 - Health Hazard Information

### Potential Health Effects

**Primary Entry Routes:** Ingestion, inhalation, eyes, skin contact.
**Target Organs:** Skin, eyes and respiratory tract.
**Acute Effects:** None reported.
**Carcinogenicity:** None of the ingredients in DeconFx Concentrate are listed as a carcinogen by the IARC, NTP or OSHA.
**Medical Conditions Aggravated by Long-Term Exposure:** None reported.
**Chronic Effects:** None reported.

### Emergency and First Aid Procedures

**Inhalation:** Remove exposed person to fresh air and support breathing as needed.
**Eye Contact:** As a general rule, flush immediately, including under eyelids, with copious amounts of water for at least 15 min.
**Ingestion:** If alert give several glasses of water or milk. Do not induce vomiting. Contact poison control center or physician.

## Section 7 - Spill, Leak, and Disposal Procedures

**Spill /Leak Procedures:** Notify safety personnel. Neutralize spills with soda ash or lime. Follow OSHA regulations (29 CFR 1910.120)
**Small Spills:** Absorb with sand or vermiculite. Sweep and collect in ordinary container and dispose. Rinse area with water.
**Large Spills** Shovel or sweep into large containers for disposal. Rinse area with water.
**Disposal:** Contact your supplier or a licensed contractor for detailed recommendations. Follow applicable Federal, state, and local regulations.
**Disposal Regulatory Requirements:** Send to a permitted waste management facility.
**Container Cleaning and Disposal:** N/A (Containers can be reused after rinsing with water.)
**OSHA Regulations:** Air Contaminant (29 CFR 1910.1000, Table Z-1, Z-1-A): Listed for phosphoric acid and hydrochloric acid.
**EPA Regulations:**

| | |
|---|---|
| RCRA Hazardous Waste Number (40 CFR 261.23): | Listed, D002, Hydrochloric Acid, Corrosive. |
| CERCLA Hazardous Waste Classification (40 CFR 302.4): | Listed, Hydrochloric Acid and Phosphoric Acid |
| CERCLA Reportable Quantity (RQ): | 5000 lb |

## Section 8 - Exposure Controls / Personal Protection

**Engineering Controls:** None
**Ventilation:** Provide general or local explosion-proof exhaust ventilation to get airborne concentration below OSHA PELs.
**Administrative Controls:** None
**Respiratory Protection:** Not required under normal conditions. If necessary, follow OSHA respirator regulations (29 CFR 1910.134) and, if necessary, wear a MSHA/NIOSH-approved respirator. If respirators are used, OSHA requires a written respiratory protection program that includes at least: medical certification, training, fit-testing, periodic environmental monitoring, maintenance, inspection, cleaning, and convenient, sanitary storage areas.
**Protective Clothing/Equipment:** For those with sensitive skin, wear protective gloves to prevent prolonged or repeated skin contact. Wear protective eyeglasses or chemical safety goggles, per OSHA eye- and face-protection regulations (29 CFR 1910.133). Contact lenses are not eye protective devices. Appropriate eye protection must be worn instead of, or in conjunction with contact lenses.
**Safety Stations:** Make emergency eyewash stations, safety/quick-drench showers, and washing facilities available in work area.
**Contaminated Equipment:** Separate contaminated work clothes from street clothes. Launder before reuse.

## Section 9 - Special Precautions and Comments

**Handling Precautions:** None
**Storage Requirements:** Store in a cool, dry, well-ventilated area away from heat, ignition sources, and incompatibles (Sec. 5).
**Comments:** Practice good personal hygiene after using this material.

## Section 10 – Transportation Information

**DOT Classification (49 CFR 173)** Hazard Class: 8 (corrosive)
Proper shipping name – Corrosive liquids, n.o.s. Packing Group: III
Identification number – UN 1760

**Prepared By:** John R. Dankert, Ph. D.

**Disclaimer:** Although reasonable care has been taken in obtaining accurate information during the preparation of this Material Safety Data Sheet, SteriFx ™, Inc. extends no warranties, express or implied, makes no representations, and assumes no responsibility as to the accuracy or suitability of such information as it applies to the purchaser's intended purpose or for consequences of its use.